

ABSTRACTS FOR POSTER PRESENTATION

Microstructural Analysis

295 POSSIBLE DOPAMINERGIC MECHANISM REGULATING THE EQUINE CORPUS LUTEUM

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Timing of embryo transfer to recipient mares is crucial to the success rate of an embryo transfer (ET) program. Recipient mares need a functional corpus luteum (CL) to maintain the early stages of pregnancy. Interference with luteal function appears to be a significant cause of failure with nonsurgical ET. Little is known about the endocrine control of luteal function in the mare, and the possibility of neuronal control of equine luteal function has not yet been studied. Dopamine (DA) has been shown to affect progesterone secretion in the bovine CL. Prior research in our laboratory suggested the possibility of dopaminergic regulation in the equine CL. The aim of this study was to document the presence of DA D1 receptor (D1r) and DA D2 receptor (D2r) within the equine CL. Immunocytochemistry (ICC) was performed on sections from 9 corpora lutea collected from a local equine abattoir. Tissues were stained using the avidin-biotin complex (ABC) method (Vectastain ABC Elite kit, Vector Laboratories, Burlingame, CA, USA). Tissues were fixed in 4% paraformaldehyde, embedded in paraffin, and cut into 5- μ m sections. Tissue sections were deparaffinized and rehydrated; endogenous peroxidase activity was quenched in 0.3% hydrogen peroxide in methanol at room temperature. To decrease nonspecific staining, tissue sections were incubated in goat serum (Vectastain ABC Elite kit). Tissue sections were then incubated overnight at 4°C with primary antibodies: Rabbit anti-Dopamine D1 receptor (Calbiochem, San Diego, CA, USA) or Rabbit anti-D2 receptor polyclonal antibody (Chemicon International, Inc, Temecula, CA, USA). Sections were then washed in PBS and incubated in biotinylated goat anti-rabbit IgG secondary antibody. After washing in PBS, sections were incubated in ABC. Sections were washed in PBS and the signal was visualized using 3-amino-9-ethylcarbazole (AEC Red, Vector Laboratories, Burlingame, CA, USA). Tissues were then counterstained with Immunomaster Hematoxylin (American Master Tech Scientific, Inc., Lodi, CA) to visualize the nuclei. As a negative control, tissues were incubated in normal rabbit serum instead of primary antibody, and as a positive control, the ICC procedure was performed on whole rat brain slices. Significant staining of luteal cells was observed using the D1r and D2r antibodies. Positive staining for D1r and D2r was seen throughout luteal cells; however, no nuclear staining was observed. The presence of these receptors in equine CL tissue suggests a functional significance for DA in luteal function. Further research needs to be performed to determine the mechanistic function of dopamine in mare reproduction.

296 LIPIDIC CONTENT IN JERSEY BLASTOCYSTS COMPARED WITH HOLSTEIN AND IVP EMBRYOS

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The objective of this study was to compare the ultrastructure of bovine embryos from different breeds and origin in terms of lipid contents. Jersey and Holstein embryos produced in vivo were obtained from superovulated donors by non-surgical method 7 days after AI. Embryos produced in vitro (Holstein cross breed) were obtained from cumulus-oocytes complexes (COC) aspirated from slaughterhouse ovaries. The COC were matured and fertilized in vitro. The zygotes were cultivated in vitro for 7 days in SOFaa media. Embryos produced in vivo (Holstein $n = 5$; Jersey $n = 5$) and in vitro ($n = 5$) classified as blastocysts grade II were fixed in Karnovsky solution immediately after embryo recovery or embryo culture and prepared for microscopic electronic evaluation. Ultrastructure of inner cell mass and trophoblast cells was analyzed. Morphometry on electron microscopy was performed using a point-count method in random samples of electron micrographs of each embryo category. The data were analyzed by chi square test. The volume density occupied by number of lipid droplets was greater in Jersey and in vitro-produced embryos compared with Holstein embryos ($24.3\% \pm 11.7$; $28.4\% \pm 19.6$ and $9\% \pm 6.68$, respectively, $P < 0,05$).